



Original Research Article

A study of antifungal susceptibility pattern of dermatophytes at tertiary care center

Siddharth Sethi^{1,*}, Ujjwal Kumar¹, Krishnendra Varma¹, Yogyata Marothi¹, Mahendra Pratap Singh Chouhan¹¹Dept. of Dermatology, Venereology and Leprology, Madhya Pradesh Medical Science University, Surasa, Ujjain, India

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ABSTRACT

Introduction: Dermatophytes are fungi that can cause infections of the skin, hair & nails due to their ability to invade keratin. Dermatophytosis is the most common superficial fungal infection worldwide; it is common in tropics and subtropical regions. It may present in epidemic proportions in areas of high humidity.

Objective: The present study aimed to identify various species causing dermatophytosis & to determine the invitro susceptibility pattern against commonly used systemic antifungal agents in our tertiary care center.

Material and Methods: A total of 149 samples were collected of infected skin, hair and nails in a period of 1 year from January 2020 to December 2020. Samples were collected under aseptic condition by skin scrapping, nail and hair clipping by using scalpel or forceps. Identification of the causative pathogen was done by performing slide culture, lacto-phenol cotton blue mount, hair perforation tests and urease tests. We adopted a newly developed agar based disk diffusion assay to test susceptibility of clinically isolated dermatophytes for antifungal susceptibility testing.

Results: Microbiological investigations revealed the presence of dermatophytic fungi in 71.8% of the samples. *Trichophyton rubrum* was the predominant pathogen isolated. The study showed Itraconazole to be most effective antifungal drugs against dermatophytes followed by terbinafine and fluconazole.

Conclusions: Further intensive epidemiological and invitro antifungal susceptibility studies of dermatophytes are required which will have more public health significance.

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1. Introduction

Dermatophytes are a homogenous group of fungi that causes superficial skin diseases in animals and humans. which obtain nutrients from keratin present in stratum corneum, hair and nails. They are important cause of superficial infections (dermatophytosis) of affecting millions of people worldwide and the risk of acquiring a dermatophyte infection in lifetime is estimated between 10–20%.¹ The species and strain of dermatophytes causing the infection determine the type and severity of the host response.

* Corresponding author.

E-mail address: drsiddharthsethi@yahoo.com (S. Sethi).

Patients who have compromised epidermis, poor hygiene, live in crowded conditions, have co-morbidities, and have close contact with people having skin and soft tissue infections are at high risk of acquiring a skin and soft tissue infections. There are several antifungal agents to treat these dermatophytes. Azole based antimycotic agents block the conversion of lanosterol to ergosterol by inhibiting enzyme lanosterol 14 α -demethylase. Finally, it disrupts structure and function of fungal membrane leading to inhibition of fungal growth. In general selection of antifungal agents will be dependent on the probable microorganisms causing infections. Some of infections respond well to topical anti-fungal agents but more extensive or severe diseases

require a systemic therapy; while in some cases, due to fungi's resistance to antifungal drugs, it is not responsive. As the availability of various antifungal drugs to treat dermatophytosis is increasing, it is important to evaluate the resistant dermatophytes using standardized, simple and reproducible in vitro assay to determine the antifungal activity of drugs against isolates. There are several methods for antifungal susceptibility of dermatophytes such as micro and macro dilution, agar dilution, E test, sensititre, colorimetric dilution and disc diffusion. All are available globally, among which dilution tests are widely used in micro and macro assays, but these methods are difficult to use in most laboratories.² The disk diffusion in vitro assay used to evaluate antifungal susceptibility testing of dermatophytes is a simple, easy to perform and economical method in developing countries which in general shows a good correlation with the reference method for micro dilution antifungal susceptibility testing.³ For evaluating the antifungal susceptibility of dermatophytes, advantages of a standardized disk diffusion-based assay includes the ease of use, reproducibility, accuracy, and low cost.^{3–5} This study was carried out to determine the antifungal susceptibilities pattern of dermatophytes from clinical specimens by using simple, inexpensive, accurate method of agar based disk diffusion assay.

2. Materials and Methods

The present study was conducted on 149 clinically diagnosed patients with dermatophytes strains belonging to 4 species, *T. rubrum* (26), *T. mentagrophyte* (18), *T. verrucosum*(2), *E. floccosum* (1) who visited department of Dermatology, R. D. Gardi Medical College (Ujjain) over a period of 1 year from January 2020 to December 2020 . The samples from patients were collected in aseptic conditions from infected areas such as skin, nail and hair.^{6,7} Specimens were processed at department of clinical Microbiology for direct microscopic examination (KOH mount) and fungal culture as per standard protocol.⁸ Culturing of organisms from skin, nail and hair was done on selective medium as Sabouraud Dextrose Agar with chloramphenicol and cycloheximide for identification and isolation of dermatophytes species.

2.1. A: Inoculum preparation

Dermatophytes were sub cultured on potato dextrose agar & incubated at 30°C for 7 days to enhance sporulation. Following the fungal growth, Culture was harvested in 1ml distilled water and colonies were probed with the help of pipette to obtain mixture of mycelium and conidia. Dense inoculum suspension of conidia and hyphal elements were transferred to sterile test tube and were allowed to sediment for 30 minutes. After the settlement of heavy particles, the upper homogeneous suspensions were transferred to another

sterile tube and were adjusted with a spectrophotometer set at 65% transmittance and 530 nm.^{9,10}

2.2. B: Disk diffusion assay

Plates of Muller Hinton Agar (MHA), with 2% glucose were inoculated using a cotton swab dipped in the standardized conidial and hyphal suspension and are exposed to air dry. The four antifungal drugs were then applied to MHA plates and after which were incubated at 28°C for 5-10 days. After the growth of colonies on plates, the sizes of zone of inhibition around the antifungal disks were measured. Antifungal criteria of sensitive, intermediate and resistance pattern of antifungal disks were reported by measuring zone of diameter in mm according to Pakshir et al.¹¹

3. Results

Table 1: Distribution of samples on the basis of KOH mount findings

Total no. of cases	KOH positive	KOH negative
149	107(71.8%)	42 (28.2%)

Table 2: Distribution of samples on the basis of culture findings

Total no. of cases	Culture positive (%)	Culture negative (%)
149	47(31.5)	102(68.5)

Out of 107 (71.8%) KOH positive samples 45 were culture positive. 42 samples were negative by KOH mount, amongst which 2 were culture positive. Total culture positivity was found in 47 cases (31.5%). Significant association between KOH mount and culture findings were observed.

Trichophyton rubrum was the most common isolate (55.3%) followed by *Trichophyton mentagrophyte* (38.3%) and *T. verrucosum* (4.2%). Only one isolate of *E. floccosum* (2.1%) was found.

In above table (Table 5) 7 strain were resistant to Fluconazole, 5 to Terbinafine, 3 and 2 to Griseofulvin and ketoconazole respectively, 18,16,6,6 and 5 strains were found intermediate sensitive to GRI, KCZ, FLC, TER and ITR respectively. Hence FLC found to be most resistant and ITR most sensitive antifungal drug.

4. Discussion

Direct microscopy with KOH was positive in 107(71.8%) cases in our study (Table 6) while 23.8% to 91.2% were reported by others (9, 13). Selection criteria of cases and the skill involved in sampling technique perhaps accounts for the difference. Hence all KOH negative samples should be cultured.

Table 3: Species of dermatophytes isolated from different clinical types (n=47)

Clinical types	T. Cruris	T.Corporis	T.Barbae	T.Capitis	T.Pedis	T.Manuum	T. Unguim	Total (%)
Isolates								
T. rubrum	15	10	-	-	1	-	-	26 (55.3%)
T.Mentagrophyte	10	6	01	-	-	01	-	18 (38.3%)
T.Verrucosum	01	-	-	-	-	-	01	02 (4.2%)
E.floccosum	01	-	-	-	-	-	-	01 (2.1%)
Total	27	16	01	-	01	01	01	47

Table 4: Interpretation of antifungal susceptibility testing, strain wise (n=47)

Isolates	Sensitive	Intermediate sensitive	Resistant
		T. rubrum (n=26)	
Itraconazole	23	3	-
Fluconazole	19	3	4
Ketoconazole	17	8	1
Griseofulvin	14	11	1
Terbinafine	20	3	3
		T. mentagrophyte (n=18)	
Itraconazole	16	2	-
Fluconazole	13	3	2
Ketoconazole	11	6	1
Griseofulvin	11	5	2
Terbinafine	13	3	2
		T. Verrucosum (n=2)	
Itraconazole	2	-	-
Fluconazole	2	-	-
Ketoconazole	1	1	-
Griseofulvin	1	1	-
Terbinafine	2	-	-
		E. Floccosum (n=1)	
Itraconazole	1	-	-
Fluconazole	-	-	1
Ketoconazole	-	1	-
Griseofulvin	-	1	-
Terbinafine	1	-	-

Table 5: Interpretation of antifungal susceptibility testing (n=47)

	Sensitive	Intermediate sensitive	Resistant
Itraconazole	42	5	0
Fluconazole	34	6	7
Ketoconazole	29	16	2
Griseofulvin	26	18	3
Terbinafine	36	6	5

Table 6: Comparison of KOH positive percentage with other studies:

Name of Author	Place	Year	Total KOH Positive Percentage
Singh S et al. ¹²	Baroda	2003	66.16%
Nada H et al. ¹³	Saudi Arabia	2005	74.08%
Amodhkumar et al. ¹⁴	Navi Mumbai	2013	78.9%
Karmakar S et al. ¹⁵	Rajasthan	1995	88.4%
Huda MM et al.	Assam	1995	92.85%

Table 7: Comparison of culture positive percentage with other studies:

Name of author	Place	Year	Total Culture Positive Percentage
Karmakar S et al. ¹⁵	Rajasthan	1995	41.6%
Singh S et al. ¹²	Baroda	2003	47.5%
Nada H et al. ¹³	Saudi Arabia	2005	53.71%
Amodhkumar et al. ¹⁴	Navi Mumbai	2013	59.09%
Belukar et al.	Thane (Mumbai)	2004	71

Table 8: Dermatophytes isolated in various studies (in percentage)

Name of the author year and place	T.rubrum	T.mentagrophytes
Fathi HI et al, 2000, Iraq. ¹⁶	20.9%	16.2%
Amodkumar et al, 2013, navi Mumbai. ¹⁴	38.46%	33.33%
Ranganathan S et al, 1995, Madras. ¹⁷	52.2%	29.35%
Bindu V et al, 2002, Calicut. ¹⁸	66.2%	25%
Venketeshan G et al, 2007, Chennai ¹⁹	73.3%	19.7%

A divergence in culture isolation ranging from 44.6 to 70.7% has been found in the Indian subcontinent. In the present study 31.5% are culture positive. However, a study by Huda MM et al done in Assam showed culture positivity of 91.66%, which was much higher and a study done at Aurangabad showed low rate of culture positivity of 22.8%.

In the present study, *T.rubrum* 26 (55.3%) was the predominant etiological agent in majority of clinical types followed by *T.mentagrophytes* 18(38.3%). High prevalence of these species and their adaptability to the Indian environment accounts for their higher isolation rates. With an increasing variety of drugs available for treatment of dermatophytes, the need for reference method for testing antifungal susceptibility of this group of fungal pathogens has become apparent.

Some studies have focused on the comparison of the disk diffusion method along with the reference micro-dilution method. These studies suggest that disk diffusion is simple, reproducible and could provide a simpler alternative for the antifungal susceptibility testing of dermatophytes in the routine clinical laboratory.

Macura²⁰ has reported that disk diffusion method of fungal susceptibility assessment yields data consistent with results obtained from the dilution method.

In this study, fluconazole showed poor activity on isolates tested (total 47).

In (7) isolates (*T. rubrum*, *T. mentagrophytes*, *T. Verrucosum* & *E. Floccosum*) no inhibition zones were observed around the disks. R. K. Agarwal et al.²¹ & other studies indicating that fluconazole had less activity against dermatophytes, which is similar to our data.

Other antifungal drug used in this study was Itraconazole which was sensitive to all (47) isolates & showed good antifungal activity. Fernandes - Torres et al.²² reported low MIC and higher IZD of Itraconazole showing good antifungal activity which is in consistent with present study.

5. Conclusion

In conclusion, itraconazole is most effective antifungal drug against dermatophytes followed by terbinafine and fluconazole is most resistant. The disk diffusion method is a simple reliable, economical and easily acceptable assay. It is more practical and easier test in comparison with dilution methods, which plays an increasingly important role in decision making for choice of drugs. However additional studies with large sample size are needed to further validate this method.

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No external funding was received to carry out this work.

7. Conflict of Interest

None.

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Author biography

Siddharth Sethi, Resident

Ujjwal Kumar, Associate Professor

Krishnendra Varma, Professor and Head

Yogyata Marothi, Professor and Head

Mahendra Pratap Singh Chouhan, Resident

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